ELECTROSPINNING AND ELECTROSPRAYING: PRODUCTION OF NANOFIBERS AND MICROPARTICLES FOR NUTRACEUTICAL / PHARMACEUTICAL APPLICATIONS

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ELECTROSPINNING AND ELECTROSPRAYING: PRODUCTION OF NANOFIBERS AND MICROPARTICLES FOR NUTRACEUTICAL / PHARMACEUTICAL APPLICATIONS

by

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To my beloved family – father Fung Kok Poy, mother Oh Ah Kim,

brother Fung Yoke Yan and sister Fung Wai Cheen

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TABLE OF CONTENTS

TITLE		i
DEDICATION		ii
ACKNOWLEDGEMENTS		iii
TABLE OF CONTENTS		iv-ix
LIST OF ABBREVIATIONS		x-xi
LIST OF TABLES		xii-xiii
LIST OF FIGURES		xiv-xv
LIST OF PLATES		xvi
LIST OF APPENDICES		xvii-xviii
ABSTRAK		xix-xx
ABSTRACT		xxi-xxii
CHAPTER 1	**INTRODUCTION**	1
1.1	Micro-Nano-Technology	1
1.1.1	Introduction	1
1.1.2	Micro-Nano-Technology Fabrication Approaches	2
	(a) Nanofibers	
	(b) Micro-Nano-Particles	
1.1.3	Electrospinning And Electrospraying	7
1.1.4	Micro-Nano-Technology In Delivery Systems	12
	(a) Micro-Nano-Particles	
	(b) Liposomes	
	(c) Carbon Nanotubes	
	(d) Nanofibers	
1.2	Agrowaste And Probiotics As Models For Electrospinning Functional Nanofibers	21
1.2.1	Introduction	21
1.2.2	Agrowaste From Oil Palm And Soy (Okara) As Functional Fiber Source	22
1.2.3	Functional Properties Of Probiotics	24

1.2.4 Preservation And Delivery Systems Of Probiotic Bacteria 27
1.3 Coenzyme Q10 (CoQ10) As A Model Drug For Drug Encapsulation 29
1.3.1 Introduction 29
1.3.2 Therapeutic Properties Of CoQ10 31
1.3.3 Bioavailability of CoQ10 32
1.3.4 Delivery Systems With Enhanced Bioavailability 34
1.4 Summary And Scope Of Study 35

CHAPTER 2 DEVELOPMENT OF ELECTROSPRAYING / ELECTROSPINNING SET-UP 37

2.1 Introduction 37
2.2 Materials 42
2.3 Methods 42
2.3.1 Electrospinning 42
(a) Instrumentation
(b) Preparation Of Electrospinning Solution And Electrospinning Process
2.3.2 Electrospraying 46
(a) Instrumentation
(b) Preparation Of Electrospraying Solution And Electrospraying Process
2.3.3 Scanning Electron Microscopy (SEM) 49
2.4 Results 49
2.4.1 Electrospinning 49
2.4.2 Electrospraying 52
2.5 Discussion 55
2.6 Conclusion 58

CHAPTER 3 UTILIZATION OF FIBER RICH RESIDUES OF AGRICULTURE WASTE IN NANO-ENCAPSULATION OF PROBIOTIC *L.* 59

ACIDOPHILUS **USING ELECTROSPINNING: FABRICATION AND CHARACTERIZATION**

3.1 Introduction 59

3.2 Materials 62

3.3 Methods 62

3.3.1 Fiber Extraction From Agrowaste 62

(a) Preparation Of Fiber Material

(b) Extraction Of Fiber Fractions

(c) Proximate Analyses

3.3.2 Electrospinning Nanofibers From Agrowaste 64

(a) Sample Preparation

(b) Electrospinning

3.3.3 Characterisation Of Electrospun Nanofibers 65

(a) Nanofiber Morphology And Size

(b) Attenuated Total Reflectance Infrared Spectroscopy (ATR-FTIR)

(c) Thermal Characterization

3.3.4 Electrospinning Agrowaste As Nano-Encapsulant For Probiotic *L. acidophilus* 66

(a) Preparation Of Stained Bacterial Suspension For Electrospinning

(b) Electrospinning

3.3.5 Characterisation Of Nanofiber-Encapsulated *L. acidophilus* 67

(a) Scanning Electron Microscopy (SEM)

(b) Fluorescence Microscopy

(c) Viability Of Assessment Of Nanofiber-Encapsulated *L. acidophilus*

3.3.6 Statistical Analysis 68

3.4 Results 69

3.4.1 Preparation Of Fiber Fractions From Agrowaste

(a) Scanning Electron Microscopy (SEM)

(b) Proximate Analyses
3.4.2 Electrospinning Nanofibers From Agrowaste 72
3.4.3 Characterisation Of Electrospun Nanofibers 74
(a) Nanofiber Morphology
(b) Attenuated Total Reflectance Infrared Spectroscopy (ATR-FTIR)
(c) Thermal Characterisation (DSC and TGA)
3.4.4 Electrospinning Agrowaste As Nano-Encapsulant For Probiotic *L. acidophilus* 81
(a) Scanning Electron Microscopy (SEM) & Fluorescence Microscopy
(b) Viability Study
3.5 Discussion 87
3.6 Conclusion 97

CHAPTER 4 PRODUCTION AND CHARACTERIZATION OF ELECTROSPRAYED MICROPARTICLES USING COQ10 AS MODEL DRUG 99

4.1 Introduction 99
4.2 Materials 103
4.3 Methods 104
4.3.1 Production Of Microparticles Using Electrospraying 104
(a) Sample Preparation
(b) Electrospraying
4.3.2 Characterisation Of Electrosprayed Microparticles 105
(a) Microparticle Morphology And Size
(b) Determination Of Yield
(c) Determination Of Water Solubility And Storage Stability
(d) X-Ray Powder Diffraction (XRPD) Analysis
(e) Differential Scanning Calorimetry (DSC) Analysis

(f) Dissolution Study
4.3.3 Statistical Analysis 108
4.4 Results 108
4.4.1 Preparation Of Electrosprayed Microparticles 108
4.4.2 Characterisation Of Electrosprayed Microparticles 110
(a) Microparticle Morphology And Size
(b) Determination Of Drug Loading And Yield
(c) Determination Of Water Solubility And Storage Stability
(d) X-Ray Powder Diffraction (XRPD) Analysis
(e) Differential Scanning Calorimetry (DSC) Analysis
(f) Dissolution Study
4.5 Discussion 124
4.6 Conclusion 138

CHAPTER 5 ***IN-VIVO*** **EVALUATION OF THE ELECTROSPRAYED COQ10 MICROPARTICLE FORMULATION IN RATS** 139

5.1 Introduction 139
5.2 Materials 141
5.3 Methods 142
5.3.1 HPLC Analysis Of COQ10 In Rat And Human Plasma 142
(a) Instrumentation
(b) Sample Preparation
(c) Standard Solutions And Calibration Curves
(d) Recovery, Precision And Accuracy
5.3.2 *In Vivo* Evaluation 144
(a) Animals
(b) Preparation Of The Oral Formulations
(c) *In Vivo* Study Design
(d) Analysis Of Plasma CoQ10

(e) Data And Pharmacokinetic Analysis
(f) Dissolution Study
5.3.3 Statistical Analysis 146
5.4 Results 147
4.4.1 HPLC Analysis Of CoQ10 In Human Plasma 147
4.4.2 *In Vivo* Evaluation 150
5.5 Discussion 155
5.6 Conclusion 160

CHAPTER 6 SUMMARY AND GENERAL CONCLUSION 161

CHAPTER 7 SUGGESTIONS FOR FURTHER WORK 164

REFERENCES 167

APPENDICES 187

LIST OF ABBREVIATIONS

ATR-FTIR	Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy
AUC	Area Under Curve
BCNU	1,3-Bis(2-Chloroethyl)-1-Nitrosourea
C.V.	Coefficient of Variation
cfu	Colony Forming Units
CNT	Carbon Nanotubes
CoQ10	Coenzyme Q10
DSC	Differential Scanning Calorimetry
EFB	Empty Fruit Bunches
FESEM	Field Emission Scanning Electron Microscope
FM	Fiber Material
FR	Fiber Residue
FS	Fiber Supernatant
HPLC	High Performance Liquid Chromatography
HPMC	Hydroxypropyl Methylcellulose
LOD	Limit Of Detection
LOQ	Limit Of Quantification
MC	Methylcellulose
MP	Electrosprayed Microparticle
MWCNT	Multi-Walled Carbon Nanotubes
NIR	Near Infra-Red
NSAID	Non-Steroidal Anti-Inflammatory Drug
OPF	Oil Palm Fronds
OPT	Oil Palm Trunks
PCL	Polycaprolactone
PEG–PLLA	Poly(Ethylene Glycol)–Poly(L-Lactic Acid)
PEO	Polyethylene Oxide
PPF	Palm Pressed Fibers
PTFE	Polytetrafluoroethylene
PVA	Polyvinyl Alcohol
RM	Raw Material
S.D.	Standard Deviation

S.E.	Standard Error
SEM	Scanning Electron Microscopy
SM	Physical Mixture
SWCNT	Single-Walled Carbon Nanotubes
TCD	Tip-Collector Distance
Tg	Glass Transition Temperature
TGA	Thermogravimetric Analysis
Tm	Melting Temperature
VA64	Copovidone Kollidon® VA64

LIST OF TABLES

2.1	Preliminary tests of electrospinning process	49
2.2	Preliminary tests of electrospraying process	53
3.1	Proximate composition of fiber materials (FM), fiber residues (FR) and fiber supernatant (FS) of oil palm trunk (OPT), oil palm frond (OPF) and okara	72
3.2	Properties of spinning solutions prepared from addition of 8 % (w/v) polyvinyl alcohol (PVA) to fiber supernatant of okara, oil palm trunk (OPT) and oil palm frond (OPF)	73
3.3	DSC Maximum of Melting (T_m), Melting Enthalpy (ΔH_m), Degree of Crystallinity (χc) of electrospun nanofibers from okara, oil palm trunk (OPT), oil palm frond (OPF) and polyvinyl alcohol (PVA)	79
3.4	Viability of *L. acidophilus* FTDC 8933 in spinning solutions (t = 01) and in nanofibers from okara, OPT and OPF stored under 4 °C, measured at time intervals t = 0, 1, 3, 5, 7, 14, 21 day(s)	85
4.1	Characteristics of the electrospray solutions and the size of electrosprayed microparticles	109
4.2	Preliminary tests of electrospraying process	109
4.3	Operating conditions used for electrospraying formulations of CoQ10:VA64 at ratios of 1:5, 1:8 and 1:10	110
4.4	Drug loading and yield of CoQ10 electrosprayed microparticles of different drug:polymer ratios (n=3, Mean ± SD)	114
4.5	Water solubility of CoQ10 in (MP) electrosprayed microparticles and (SM) physical mixture formulations (n=3, Mean ± SD)	115
4.6	Storage stability of CoQ10 solubility in 1:8 (CoQ10:VA64) electrosprayed microparticles (n=3, Mean ± SD)	116
4.7	DSC Maximum of Melting (T_m), Melting Enthalpy (ΔH_m), Degree of Crystallinity (χc) of (RM) CoQ10 raw material; (SM) physical mixture of CoQ10:VA64 (1:8) and (MP) electrosprayed particles of CoQ10:VA64 (1:8)	119
5.1	Sequence of administration of the CoQ10 raw material, physical mix and electrosprayed microparticles	145
5.2	Regression parameters and statistics for calibration curves (n=6) of the human plasma CoQ10 assay method	149

5.3 Absolute recovery, within-day, and between-day accuracy and precision (n=6) of human plasma CoQ10 HPLC assay method 149

5.4 The C_{max}, T_{max} and AUC_{0-120h} of plasma CoQ10 in rats following 400mg/kg oral dosing in the form of raw material (RM), physical mixture (SM) and electrosprayed microparticles (MP) 152

5.5 The 90 % confidence intervals of the AUC_{0-120h} and C_{max} values of plasma CoQ10 following oral administration in the form of raw material (RM), physical mixture (SM) and electrosprayed microparticles (MP) 154

LIST OF FIGURES

1.1	Nanofiber fabrication techniques (a) Phase separation, (b) Self assembly, (c) Drawing and (d) Template synthesis Nanofiber fabrication techniques (A) Phase separation, (B) Self assembly, (C) Drawing and (D) Template synthesis (adapted from Figures 1-4, Pp. 56-57, Kuan, et al., 2012, Nanotech: Propensity in Foods and Bioactives. Crit Rev in Food Sci Nutri 52: 55-71)	4
1.2	Illustrations of electrospinning (adapted from Figure 1. The common setup and working principle of electrospinning, page 420, Li, et al., 2010. Core-Shell Nanofibers: Nano Channel and Capsule by Coaxial Electrospinning. In: Nanofibers, Ed: Ashok Kumar)	9
1.3	Illustrations of electrospraying (adapted from Figure 1. Schematic of the electrospray crystallization process, page 1, Ambrus, et al., 2013, Analysis of submicron-sized niflumic acid crystals prepared by electrospray crystallisation. Journal of Pharmaceutical and Biomedical Analysis 76: 1-7)	10
1.4	Characteristic spray-modes found during electrohydrodynamic process (adapted from Figure 2. Characteristic spray-modes found during electrohydrodynamic atomization of polymer solutions, page 69, Hogan et al., 2007, Controlled size polymer particle production via electrohydrodynamic atomization. Colloids and Surfaces A: Physicochemical and Engineering Aspects 311: 67-76).	11
1.5	Chemical structure of CoQ10	30
2.1	Coaxial electrospraying setup for production of drug loaded particles (adapted from Figure 1. Experimental set-up used for the production of drug-loaded particles, Page 3, Enayati, et al., 2010, One-step electrohydrodynamic production of drug-loaded micro- and nanoparticles. J. R. Soc. Interface 7(45): 667-675)	38
2.2	Electrospraying unit with nitrogen gas inlet to aid solvent evaporation (adapted from Figure 1. Schematic of the system used for electrohydrodynamic atomization, page 68, Hogan et al., 2007, Controlled size polymer particle production via electrohydrodynamic atomization. Colloids and Surfaces A: Physicochemical and Engineering Aspects 311: 67-76)	39
3.1	ATR-FTIR spectra of electrospun nanofibers from polyvinyl alcohol (PVA), okara, oil palm trunk (OPT) and oil palm frond (OPF)	76
3.2	TGA thermogram of electrospun nanofibers from polyvinyl alcohol (PVA), okara, oil palm trunk (OPT) and oil palm frond (OPF)	78
3.3	DSC thermogram of electrospun nanofibers from polyvinyl alcohol (PVA), okara, oil palm trunk (OPT) and oil palm frond (OPF)	80

3.4 Preservation of cell viability (% viability retained) of *L. acidophilus* FTDC 8933 in spinning solutions (t = 01) and in nanofibers from okara, OPT and OPF stored under 4 °C, measured at time intervals t = 0 (t = 02), 1, 3, 5, 7, 14, 21 day(s); n=4; Mean ± Standard error 86

3.5 Schematic illustration of rod-like bacteria at the sink-like flow. The bacteria are gradually oriented among the stream lines (adapted from Figure 2. Schematic illustration of rod-like bacteria at the sink-like flow, Page 4676, Salalha, et al., 2006, Encapsulation of bacteria and viruses in electrospun nanofibres. Nanotechnology 17: 4675-4681) 94

4.1 X-ray diffraction patterns of (RM) CoQ10 raw material; (SM) physical mixture of CoQ10:VA64 (1:8) and (MP) electrosprayed microparticles of CoQ10:VA64 (1:8) 117

4.2 DSC thermographs of (A) CoQ10 raw material; (B) copovidone VA64; (C) physical mixture of CoQ10:VA64 (1:8) and (D) electrosprayed microparticles of CoQ10:VA64 (1:8) 120-121

4.3 Dissolution versus time profiles of CoQ10 in the form of raw material (RM), physical mixture of CoQ10:VA64 (1:8) (SM) and electrosprayed microparticles of CoQ10:VA64 (1:8) (MP) (n=6, Mean ± SE) 123

5.1 Chromatograms for the analysis of CoQ10 in (a) blank human plasma spiked with 5000 ng/mL CoQ10 and (b) rat plasma containing 1924 ng/mL of CoQ10 obtained 48 hours following oral administration of 400 mg/kg of CoQ10 in electrosprayed microparticle formulation. Retention time of CoQ10 was approximately 5.2 mins. 148

5.2 Mean plasma concentration (ng/mL) versus time profiles of CoQ10 following single oral dosing of 400mg/kg in the form of raw material (RM), physical mixture (SM) and electrosprayed microparticles (MP) 151

LIST OF PLATES

2.1	The Electrospinz electrospinning setup	43
2.2	The electrospinning setup after slight modification	43
2.3	The self-assembled electrospinning setup	44
2.4	The electrospraying setup	47
2.5	The nanofibers of 5 % (w/v) PVA electrospun at (A) 8 kV and tip-collector distance (TCD) of 10 cm; (B) 10 kV and TCD of 15 cm; (C) 15 kV and TCD of 15cm; and 8 % (w/v) PVA electrospun at (D) 10 kV and TCD of 15 cm; (E) 12 kV and TCD of 15 cm	51-52
2.6	The microparticles of 1 % (w/v) A15:E5 (1:1) electrosprayed at (A) 15 kV and tip-collector distance (TCD) of 5 cm; (B) 20 kV and TCD of 10 cm; and (C) 25 kV and TCD of 20cm	54
3.1	SEM micrographs of fiber materials (FM) and fiber residues (FR) of (A) okara, (B) oil palm trunk and (C) oil palm frond	70
3.2	SEM micrographs of electrospun nanofibers from agrowaste fiber supernatant of (A) okara, (B) oil palm trunk (OPT) and (C) oil palm frond (OPF)	75
3.3	SEM micrographs of nanofibers containing *L. acidophilus* from electrospinning FS of (A) okara, (B) oil palm trunk (OPT) and (C) oil palm frond (OPF)	82
3.4	Optical micrographs of *L. acidophilus*-containing nanofibers using fluoresecence illumination with FDA revealing live bacteria within the fibers	83
4.1	Digital microscope images of (A) CoQ10 raw material; (B) physical mixture of CoQ10:VA64 (1:8) and (C) electrosprayed microparticles of CoQ10:VA64 (1:8)	111
4.2	SEM micrographs of CoQ10 (A) raw material and (B) physical mixture	112
4.3	SEM micrographs of electrosprayed microparticles of CoQ10:VA64 (A) 1:5; (B) 1:8 and (C) 1:10	113

LIST OF APPENDICES

3.1(a) Proximate composition of fiber materials (FM) of okara

3.1(b) Proximate composition of fiber materials (FM) of oil palm trunk (OPT)

3.1(c) Proximate composition of fiber materials (FM) of oil palm frond (OPF)

3.2(a) Proximate composition of fiber residues (FR) of okara

3.2(b) Proximate composition of fiber residues (FR) of oil palm trunk (OPT)

3.2(c) Proximate composition of fiber residues (FR) of oil palm frond (OPF)

3.3(a) Proximate composition of fiber supernatant (FS) of okara

3.3(b) Proximate composition of fiber supernatant (FS) of oil palm trunk (OPT)

3.3(c) Proximate composition of fiber supernatant (FS) of oil palm frond (OPF)

4.1 Conductivity (µS/cm) of electrospraying solutions

4.2 Surface tension (N/m) of electrospraying solutions

4.3 Viscosity (mPa.S) of electrospraying solutions

4.4 Drug loading of CoQ10 electrosprayed microparticles of different drug:polymer ratios

4.5 Yield of CoQ10 electrosprayed microparticles of different drug:polymer ratios

4.6 Water solubility of CoQ10 in (MP) electrosprayed microparticles and (SM) physical mixture formulations of different drug:polymer ratios

4.7 Storage stability of CoQ10 solubility in 1:8 (CoQ10:VA64) electrosprayed microparticles

4.8(a) Dissolution of CoQ10 in the form of raw material (RM)

4.8(b) Dissolution of CoQ10 in the form of physical mixture of CoQ10:VA64 (1:8) (SM)

4.8(c) Dissolution of CoQ10 in the form of electrosprayed microparticles of CoQ10:VA64 (1:8) (MP)

5.1 Calibration curve of CoQ10 in human spiked plasma (n=6)

5.2(a) Plasma CoQ10 concentration of individual rats after oral administration of CoQ10 raw material (RM)

5.2(b) Plasma CoQ10 concentration of individual rats after oral administration of CoQ10 physical mixture (SM) formulation

5.2(c) Plasma CoQ10 concentration of individual rats after oral administration of CoQ10 electrosprayed microparticle (MP) formulation

5.3 Animal Ethics Committee Approval Letter

PEMUTARAN-ELEKTRO DAN PENYEMBURAN-ELEKTRO: PENGHASILAN GENTIAN-NANO DAN ZARAH-MIKRO UNTUK APLIKASI NUTRASEUTIK / FARMASEUTIK

ABSTRAK

Kajian ini menyiasat teknologi pemutaran-elektro dan penyemburan-elektro dalam pengeluaran dan aplikasi struktur nano dan mikro. Satu unit pemutaran-elektro mudah telah dibina untuk pemutaran-elektro dan satu unit yang sama telah disesuaikan dengan komponen tambahan untuk menampung keperluan yang berbeza untuk pemutaran-elektro. Persediaan unit pemutaran-elektro kemudiannya digunakan dalam pengeluaran gentian-nano daripada sisa agro, iaitu batang kelapa sawit (OPT), pelepah kelapa sawit (OPF) dan okara (sisa pepejal kacang soya) sebagai pembawa bakteria probiotik *L. acidophilus*. Serat terlarut dan hemiselulosa diperolehi daripada rawatan alkali sisa agro ditambah dengan polyvinil alkohol 8 % (w/v) dan diputar menjadi gentian nano yang seragam saiznya. Sel bacteria *L. acidophilus* dicampurkan ke dalam larutan itu dan diputar menjadi gentian-nano yang mengandungi bakteria probiotik. Bakteria masih hidup walaupun dikenakan tegasan ricih dan voltan tinggi semasa pemutaran-elektro dan mengekalkan daya maju semasa penyimpanan dalam suhu peti sejuk (4 ° C). Selepas penyimpanan selama 21 hari pada suhu sejuk (4 º C), *L. acidophilus* terkandung dalam gentian-nano OPF mengekalkan sehingga 71.9 ± 1.0% sel-sel berdaya maju berbanding dengan sampel segar. Teknologi penyemburan-elektro digunakan untuk menyediakan formulasi ubiquinone (CoQ10), drug BCS Kelas II (kelarutan yang rendah, kebolehtelapan yang tinggi) untuk meningkatkan sifat keterlarutan dan biokeperolehan oral. Copovidone VA64 dicampur dengan CoQ10

(1:5, 1:08 dan 1:10 CoQ10:VA64) untuk menghasilkan zarah-mikro. Zarah-mikro amorf yang licin permukaannya dan bersaiz dalam lingkungan 3 hingga 5 μm, menunjukkan keterlarutan air yang lebih tinggi berbanding dengan bahan mentah CoQ10. Ini dikaitkan dengan perubahan dalam keadaan hablur CoQ10. Penilaian *in vitro* zarah-mikro menunjukkan sifat-sifat meningkatkan biokeperolehan seperti pengurangan penghabluran, pengurangan saiz zarah, dan peningkatan keterlarutan dalam air. Hal ini dibuktikan lagi dengan kajian *in vivo* menggunakan model tikus. Profil penyerapan jelas menunjukkan bahawa formulasi zarah-mikro mencapai tahap plasma tertinggi diikuti oleh campuran fizikal, manakala bahan mentah menunjukkan tahap plasma purata CoQ10 yang paling rendah. Tahap penyerapan zarah-mikro adalah kira-kira 2.9 kali lebih tinggi daripada bahan mentah CoQ10, dan kira-kira 1.6 kali lebih tinggi daripada formulasi campuran fizikal. Berdasarkan keputusan kajian ini, pemutaran-elektro dan penyemburan-elektro merupakan teknologi berpotensi tinggi untuk menghasilkan struktur-nano dan -mikro sebagai mod penghantaran untuk bahan bioaktif seperti bakteria probiotik dan ubat-ubatan dengan biokeperolehan oral yang rendah. Kemantapan dan fleksibiliti teknologi boleh dioptimumkan untuk menghasilkan struktur-nano dengan pelbagai ciri, sesuai untuk fungsi-fungsi spesifik.

ELECTROSPINNING AND ELECTROSPRAYING: PRODUCTION OF NANOFIBERS AND MICROPARTICLES FOR NUTRACEUTICAL / PHARMACEUTICAL APPLICATIONS

ABSTRACT

The present study investigated the technology of electrospinning and electrospraying in the production and application of nano- and micro-structures. A simple bench-top setup was constructed for electrospinning and a similar unit was adapted with additional components to accommodate the different requirement of electrospraying. The electrospinning setup was subsequently used in the production of functional nanofibers from agricultural waste materials, namely oil palm trunk (OPT), oil palm frond (OPF) and okara (solid soy waste) as encapsulants for probiotic bacteria *L. acidophilus.* Solubilised soluble fibers and hemicelluloses obtained from alkaline treatment of the agrowaste was added with polyvinyl alcohol 8 % (w/v) and successfully electrospun into uniform-sized nanofibers. *L. acidophilus* was added into the solution and electrospun into nanofiber encapsulated probiotic bacteria. The bacteria survived the electrospinning conditions of high shear stress and high voltage and remained viable during storage at refrigeration temperature (4 °C). OPF nanofiber-encapsulated *L. acidophilus* stored at refrigerated temperature (4 ºC), retained up to 71.9 ± 1.0 % of viable cells compared to fresh samples after 21 day storage. The electrospraying technology was applied to prepare ubiquinone (CoQ10), a BCS Class II drug (low solubility and high permeability) to enhance its dissolution properties and oral bioavailability. Copovidone VA64 was formulated with CoQ10 in various ratios (1:5, 1:8 and 1:10 of CoQ10:VA64) to form polymeric microparticles. The smooth

amorphous microparticles in the range of 3 to 5 µm, showed enhanced water solubility, which could be attributed to the change in crystalline state of CoQ10. *In vitro* evaluation of the electrosprayed microparticles showed bioavailability enhancing properties such as reduced crystallinity, reduced particle size, and increased water solubility. This was further affirmed in *in vivo* study using rat model. The absorption profiles clearly show that the electrosprayed microparticle preparation achieved the highest plasma levels followed by the physical mixture, while the raw material produced the lowest mean plasma levels of CoQ10. The extent of absorption of the microparticles was approximately 2.9 times higher than that of the raw material CoQ10, and approximately 1.6 times over that of the physical mixture formulation. On the basis of the findings in this study, electrospinning and electrospraying are highly prospective technologies to produce functional nano- and micro-structures as delivery vehicles for bioactives such as probiotic bacteria and drugs with poor oral bioavailability. The robustness and flexibility of the technology can be optimised to produce functional nanostructures with a wide range of properties, suited for specific functions.

CHAPTER 1: INTRODUCTION

1.1 MICRO-NANO-TECHNOLOGY

1.1.1 INTRODUCTION

Micro-technology refers to the fabrication and application of materials in the dimensions of 1 to 1000 μm, irrespective of the precise interior or exterior structure. In micro-technology and micro-engineering, the most widely researched application would be microparticles. Microparticles, encompass two structural subtypes, namely "microspheres" which are spherical microparticles and "microcapsules" which are microparticles with a distinctive core surrounded by an outer encapsulant (Singh et al., 2010, Birnbaum et al., 2003). Nanotechnology is the application of science and engineering to fabricate structures of dimensions in the nanometer scale (Nalwa, 2000).

Nanotechnology, first coined by Japanese engineer Norio Taniguchi in 1974, originally implied the control of materials on the order of nanometers, more specifically in the range of 1 – 100 nm, and has since developed into a science of intentional creation, manipulation and characterisation of the macro-molecules (Dingman, 2008; Resnik & Tinkle, 2007). The sub-micron dimensions give rise to unique shape, orientation, surface chemistry, reactivity and topology, which are translated into unusual electrical, optical, magnetic, mechanical, thermal and biological properties for these materials (Laurencin & Nair, 2008). However, challenges persist at controlling the morphology, structure, composition, and size of nanomaterials; factors which define the unique physical properties of the resulting materials (Lieber, 2003). Increasing knowledge in biocompatible polymers such as

polysaccharides, lipids and biopolyesters has spurred advances in the application of micro-nano-technology in functional food and drug delivery systems.

1.1.2 MICRO-NANO-TECHNOLOGY FABRICATION APPROACHES

The fabrication of micro-nano-materials can be broadly distinguished into two general pathways, namely top-down and bottom-up. The top-down approach involves reducing large materials or structures using various physical or physicochemical methods down to micro- or nano-dimensions. This approach may result in costly sample loss in the disposal of excess materials. Meanwhile, the bottom-up approach involves building structures up to the desired micro- or nano-dimensions from the components' atomic or molecular phases. While this could be time consuming, the bottom-up approach is generally preferred due to greater energy efficiency and better control of the structure and properties of nanomaterials formed (Viney, 2004). Examples of top-down and bottom-up fabrication methods are grinding or milling, and nanoemulsion formation, respectively. There are other fabrications methods in both approaches, which are continuously being refined to achieve the desired properties for distinct applications in food and drugs.

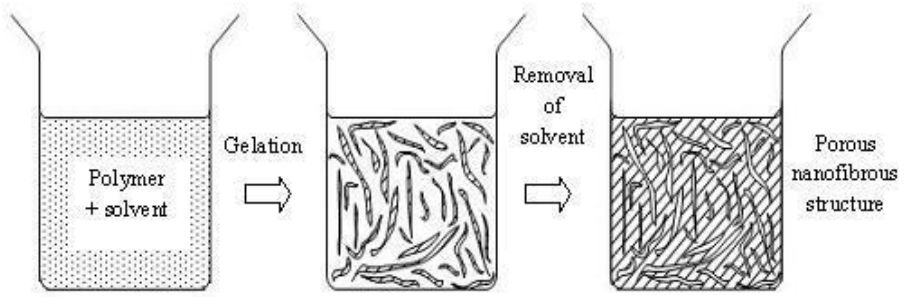
a) Nanofibers

Nanofibers are commonly fabricated using methods such as phase separation, self assembly, template synthesis, drawing and electrospinning. Phase separation technique (Figure 1.2 A) applies polymer dissolution, gelation, and solvent extraction to produce porous nanofibrous structure. Dissolved polymers undergo gelation, leading to the production of two physically incompatible phases, and subsequently phase separation. The gel phase separated from the solvent phase is freeze-dried to

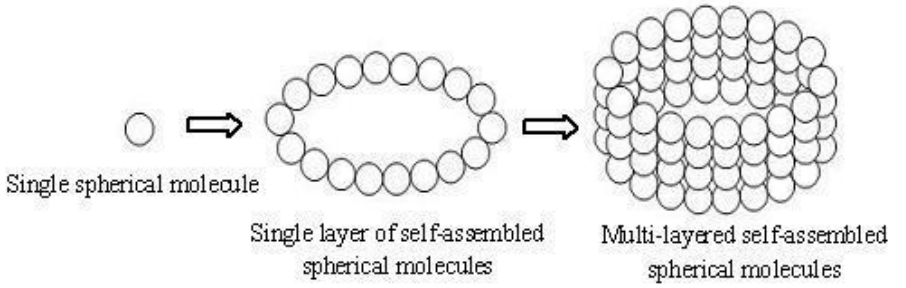
obtain porous nanofibrous structure. Mechanical properties of the nanofibers can be engineered to suit specific applications by varying the polymer type and concentration used (Ramakrishna et al., 2005). Commonly used polymers to fabricate nanofibers using the phase separation technique include biodegradable polymers such as poly(L-lactic) acid (PLLA) and its derivatives (Kuan et al., 2012).

The self-assembly technique (Figure 1.2 B) involves the spontaneous self-arrangement of small building molecules under appropriate intermolecular forces, which can be achieved by manipulation of the physical and chemical conditions such as pH, temperature and solute concentrations. The basic building blocks and the strength of the intermolecular forces dictate the morphology of the formed nanofibers (Kuan et al., 2012). Biomolecules such as proteins, peptides and DNA have been processed via self-assembly to form micro-nano-structures used in tissue cell cultures (Rajagopal & Schneider, 2004; Zhang et al., 2004; Doyle, 2006).

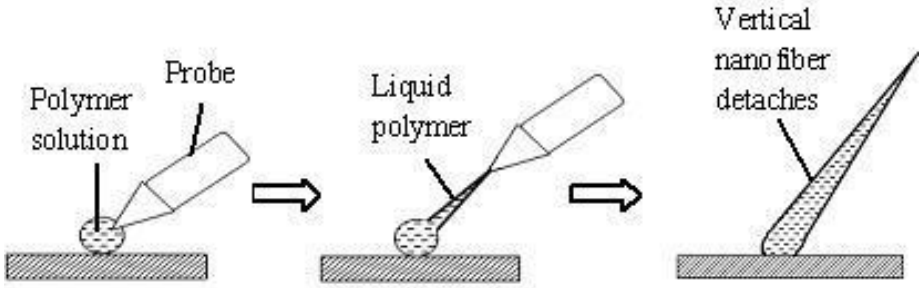
Drawing is used to synthesise micro-nano-fibers by drawing out liquid droplet with a diameter of a few micrometers using a micropipette (Figure 1.2 C). The drawing process is repeated several times on every droplet and pulled fibers are deposited on the surface by touching it with the end of a micropipette (Kuan et al., 2012). Although tedious, the technique produces long single micro-nano-fibers via a simple process requiring minimal equipment. Polystyrene nanofibers for biological scaffoldings were produced using this technique in a study by Nain et al. (2006).



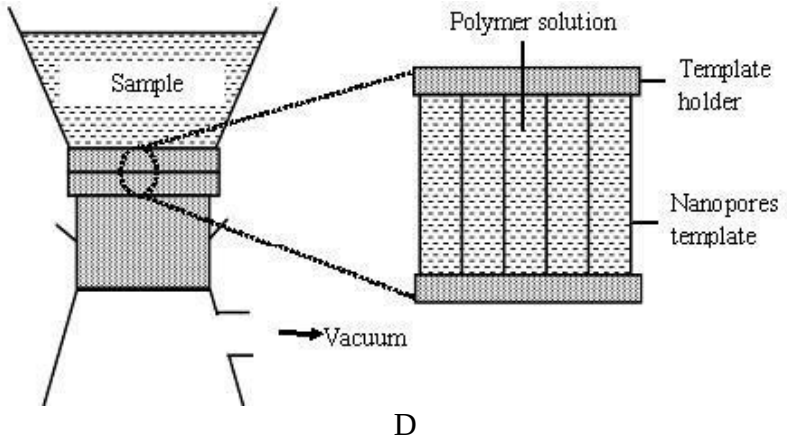
A



B



C



D

Figure 1.1 Nanofiber fabrication techniques (A) Phase separation, (B) Self assembly, (C) Drawing and (D) Template synthesis (adapted from Figures 1-4, Pp. 56-57, Kuan et al., 2012, Nanotech: Propensity in Foods and Bioactives. Crit Rev in Food Sci Nutri 52: 55-71)

Template synthesis (Figure 1.2 D) is extensively used in the production various types of nanofibers such as hollow nanofibers, composite nanowires and segmented nanowires. The nanofiber diameters are determined by the size of the pores on the template (Ramakrishna et al., 2005; Kuan et al., 2012). The fundamentals and various technical approaches developed for the template-based synthesis of nanorod, nanowire and nanotube arrays was reviewed by Cao & Liu (2008).

b) Micro-nano-particles

Micro-nano-particles can be fabricated using mechanical and physicochemical methods such as grinding, emulsification, solvent evaporation, spray drying and coacervation. In mechanical grinding, large structures or polymers are milled down using mechanical force to smaller size particles. The morphology and size of the micro-nano-particles can be controlled by milling time and the force applied. Thin drug-containing ethylcellulose films in cryogenic conditions were milled to form drug encapsulated microparticles whereby the particle shape and size where also dictated by the thickness of the film (Oliveira & Mano, 2011).

Spray drying is another physical approach which is widely used in the preparation of pharmaceuticals and microencapsulation. The method involves dispersing a drug or polymer solution into continuous stream of hot air to vaporise drug solutions, producing dried micro-nano-particles. The size of the particles is dependent on several parameters such as polymer/drug solution loading rate, drying temperature, and spraying rate (Elversson & Millqvist-Fureby, 2005; Oliveira & Mano, 2011). However, the utilisation of high temperature in this process limits the application to heat-stable compounds.

Emulsification is one of the most commonly used approaches for micro-nano-particle fabrication. The approach is often coupled with other strategies such as solvent evaporation and salting out to form particles of desired properties. In emulsification, polymers or drugs are dissolved in a solvent, to which another solution which is non-miscible is added. Pure polymeric micro-nano-particles are usually produced by a single oil-in-water (o/w) emulsion (Oliveira & Mano, 2011). Emulsifiers or surfactants are often used, together with mechanical force such as vigorous homogenization, to facilitate the formation of micro-nano-droplets within the emulsion.

In salting out technique, the addition of water or aqueous solution to the emulsion causes the diffusion of organic solvent into the aqueous phase, simultaneously aggregating the water insoluble polymer and bioactive compounds to form micro-nano-particles (Weiss et al., 2006). The phase separation causes the polymer and bioactive compounds to precipitate into micro-nano-spheres, which is collected by evaporating the volatile solvent under reduced pressure conditions or continuous stirring (Weiss et al., 2006). Salting out minimises stress to protein encapsulants and does not require high processing temperature, thus it is useful in processing heat sensitive substances (Zohri et al., 2009). In solvent evaporation, immiscible volatile organic solvent is added to an aqueous solution containing surfactant and homogenised to form an emulsion, followed by the evaporation of the solvent to collect the resulting micro-nano-particles. This method is has been applied to produce micro-nano-particles containing albumin, testosterone and cyclosporine A (Zohri et al., 2009). Nanoliposomes have also been fabricated by dissolving in organic solvents followed by removal of the solvent via evaporation and hydration in aqueous buffers (Wu et al., 2007; Kuan et al., 2012).

Despite the ease of micro-nano-particle formation using emulsification techniques, the use of solvents in the production of micro-nano-particles may limit its application in food and pharmaceuticals. Hence, other methods with better solvent removal technique or minimal use of solvents have been proposed and applied, such as coacervation, ionic gelation, super critical technology and polymerisation (Ochekpe et al., 2009). A new technique supercritical fluid mixing involves spraying a polymer/bioactive solution into supercritical fluid which results in the dissolution of the solvent in the supercritical phase and precipitation of the micro-nano-particles (Oliveira & Mano, 2011). The resulting product is solvent free in the range of 1 – 120 μm, depending on the properties of polymers used such as molecular weight, crystal structure, glass transition temperature, and solubility (Oliveira & Mano, 2011).

Most of these methods have some disadvantages such as low drug-loading efficiency, limitations to scale up, particle-size polydispersity, low ability to fabricate small particles (below 100 nm), and difficulties for incorporation of hydrophilic drugs (Zamani et al., 2013). Moreover, the usage of organic solvents, high shear stress and high temperature, may cause the degradation of bioactive compounds which are used in the design of delivery systems (Zamani et al., 2013).

1.1.3 ELECTROSPINNING AND ELECTROSPRAYING

A novel technology which may serve as an alternative to the conventional micro-nano-structure fabrication methods is the electrohydrodynamic techniques. The technology encompasses electrospinning and electrospraying in the production of fibers and particles, respectively, often in sub-micron dimensions.

The electrohydrodynamic technology utilises electrostatic force to draw out polymeric solutions from a fine orifice. Depending on the properties of the solution and operation parameters, the drawn solution droplets are spun into fibers or sprayed into particles. The subsequent rapid evaporation process results in dry, solvent-free structures which have been reduced to nano-size. The quality and characteristics of the final product are determined by the elasticity, viscosity, temperature, humidity, conductivity and surface tension of the polymer, strength of the electric field, and the distance between the orifice and collector (Heunis et al, 2010). Thus, the size and properties of nanofibers or micro-nano-particles can be designed to suit specific applications by modifying the parameters of the spinning process (voltage, spinning distance, flow rate) and that of the spinning solution (viscosity, conductivity, surface tension) (Munir et al., 2009; Lopez-Rubio et al., 2009; Rezvanpour et al., 2010).

Electrospinning into nanofibers occurs when polymer solutions of high chain entanglements and elasticity are used. The network of entanglements enables the solution to remain elastic and elongate during the whipping motion of the electrically-charged fluid jet. Electrospun nanofibers from synthetic and natural polymers such as polyamide, nylon, polyethylene oxide, protein, peptides, and carbohydrates have been applied in drug delivery system, tissue engineering and functional material development (Ramakrishna et al., 2005; Lu & Ding, 2008; Kuan et al., 2012).

Electrospraying or electrohydrodynamic atomisation applies the same principles of electrospinning to produce micro-nano-particles from solutions of high surface tension and low chain entanglements. The technique can be used to fabricate particles with narrow size distribution, ranging from 200 μm to 2 mm (Oliveira & Mano, 2011).

When the solution droplet of low chain entanglements is electrically charged, it deforms and disrupts into droplets instead of elongated fibers. The rapid evaporation of solvent and further particle fission reduces the size of the droplets into dry microparticles upon deposition onto the grounded collector. Electrospraying has the advantage of higher loading efficiency, narrow particle-size distribution, and ease of particle synthesis due to single-step processing (Zamani et al., 2013).

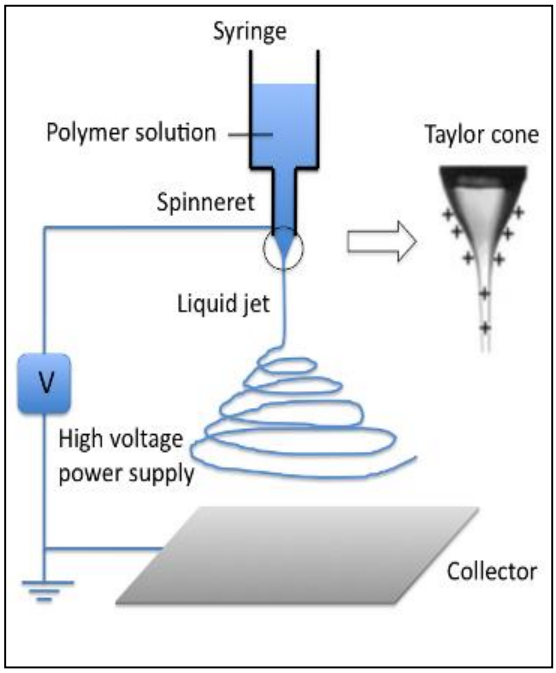


Figure 1.2 Illustrations of electrospinning (adapted from Figure 1. The common setup and working principle of electrospinning, page 420, Li et al., 2010, Core-Shell Nanofibers: Nano Channel and Capsule by Coaxial Electrospinning. In: Nanofibers, Ed: Ashok Kumar)

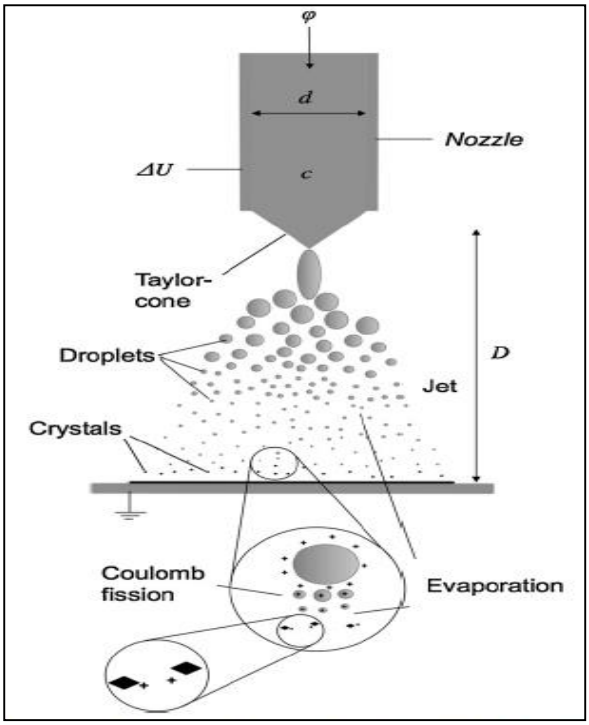


Figure 1.3 Illustrations of electrospraying (adapted from Figure 1. Schematic of the electrospray crystallization process, page 1, Ambrus et al., 2013, Analysis of submicron-sized niflumic acid crystals prepared by electrospray crystallisation. Journal of Pharmaceutical and Biomedical Analysis 76: 1-7)

Since electrospraying was first systematically studied by Zeleny (1914) and theoretical explained by Taylor (1964), many researchers have investigated the mechanisms that induce an electric charge on the surface of a liquid and stretch the meniscus in the direction of the electric field (Grafahrend et al., 2010). As such, several modes have been observed on the droplets drawn into the electrospining or electrospraying process (Figure 1.4). The most stable mode observed is the cone-jet mode, which when achieved, results in a stable electrospinning or electrospraying process and monodispersed structures. However, with the increasing strength of applied voltage, four other operation modes were observed, namely the dripping mode, silver-bullet mode, compound cone-jet mode (if possible), and pulsated cone-jet mode (or multi-jet mode) (Mei & Chen, 2008). The cone jet mode only occurs when the liquid meniscus assumes the shape of a sharp cone, obtained by balancing the electrostatic

and capillary pressure acting on a conical surface for ideal, static equilibrium conditions (Grafahrend et al., 2010).

Figure 1.4 Characteristic spray-modes found during electrohydrodynamic process (adapted from Figure 2. Characteristic spray-modes found during electrohydrodynamic atomization of polymer solutions, page 69, Hogan et al., 2007, Controlled size polymer particle production via electrohydrodynamic atomization. Colloids and Surfaces A: Physicochemical and Engineering Aspects 311(1–3): 67-76).

The resulting micro-nano-particles and nanofibers fabricated using electrohydrodynamic processes have been applied in various fields such as textile, construction, biotechnology, food, medical and pharmaceutical. While the use of micro-nano-particles and nanofibers in the medical field started with drug delivery, the application has promptly expanded into tissue engineering, with vast literature on various biocompatible synthetic or natural polymers being used. The use of nanofibers and micro-nano-particles can be justified for the following pharmaceutical applications: prolonged release dosage forms, enteric-coated dosage forms for selective release in the digestive tract, masking the bitter taste drugs, aid in the addition of oily medicines to tableted dosage forms, protect drugs from environmental hazards such as humidity, light, oxygen or heat, decrease contact with toxic or noxious substances, and increase the absorption efficiency of the encapsulated bioactive (Singh et al., 2010).

1.1.4 MICRO-NANO-TECHNOLOGY IN DELIVERY SYSTEMS

The development of delivery systems for biologically active compounds is an important issue in modern food and pharmaceutical technology. The term "microencapsulation" designates a defined technology of wrapping solids, liquids, or gases in small capsules, which can release their contents under specific circumstances (Lopez-Rubio et al, 2009). Micro-nano-technology is widely applied in food and pharmaceutical sciences, especially in the design of delivery systems. Since both nano- and micro scale systems have shown critical advantages in developing various clinically useful drug delivery systems, thus "nanotechnology" includes "microtechnology," and "nanofabrication" or "nanomanufacturing" and its micro counterparts (Park, 2007). In drug delivery systems, applicable dimensions typically range from truly nanosystems to microparticles in the range of 100 μm (Park, 2007). Meanwhile, nanotechnology generally affects the food industry in the development of functional food delivery system, food packaging, food security and molecular synthesis of new functional food compounds (Moraru et al., 2003).

Drug delivery emphasises on transport and targeted delivery of drugs to desirable sites, while concurrently minimising side effects on other tissues (Tran et al., 2009). Controlled delivery systems via micro-nano-particles improve the therapeutic efficacy and safety of drugs (Vasita & Katti, 2006). Micro-nano-structures are able to exploit biological pathways and provide unprecedented advantage of size by delivering proteins, drugs and genetic material to previously inaccessible cellular and intracellular targets, leading to greater therapeutic benefits and reduced side effects, due to its selective sub-cellular delivery (Faraji & Wipf, 2009). The greater surface area of micro-nano-particles also gives rise to enhanced bioactivity compared to their

larger-sized counterparts. Nanoparticles are also able to establish sustained-release drug profiles and prolong compound residence time in the gastrointestinal tract, thus providing efficient delivery of compounds to targeted sites (Chau et al., 2007).

The application of nanofibers in delivery systems and tissue engineering is highly feasible due to the remarkable characteristics such as the high surface area-to-volume ratio, high interconnected porosity with tunable pore size, possibilities for efficient surface functionalization, adjustable surface morphology, and structural similarity to the extracellular matrix (Oliveira & Mano, 2011). As for microparticles, the size polydispersity is often the constraint limiting the potential application in drug-delivery system, especially inhalable drugs where the aerosol particles with homogeneous size and suitable aerodynamic diameter (1–5 μm) are essential (Zamani et al., 2013).

(a) Micro-Nano-particles

Micro-nano-particles present various advantages over conventional drug delivery systems, including effective protection of the encapsulated bioactive agent against degradation, controlled or sustained release, targeted release, easy administration and reduced toxicity or irritancy due to lowered local concentrations of drug (Singh et al., 2010). Singh et al. (2010) reviewed the role of micro-nano-particles as drug delivery devices for increasing efficiency of drug delivery, improving the release profile and drug targeting.

Micro-nano-particles deliver drugs, which are chemically bound, adsorbed or entrapped, via inhalation, oral or intravenous routes. Polyethylene glycol (PEG)-coated (PEGylated) nanospheres used in intravenous drug delivery is enhanced with

protection against blood components and plasma proteins, preventing its removal from the blood and prolonging systemic circulation (Jagur-Grodzinski, 1999). PEGylation, a standard strategy in in-vivo drug targeting, provides stealthing of nanoparticles, or invisibility to macrophages and phagocytes, hence prolonging its systemic half-life. Nanoparticles are also suitable for intravenous delivery as they are small enough to safely pass through the body's smallest vessels, and have increased surfaced area for rapid dissolution (Cohen-Sela et al., 2007).

One of the largest applications for drug delivery is in cancer treatment where a proper amount of drugs needs to be directed to the targeted tissue while minimising unwanted effects on normal, healthy tissue (Tran et al., 2009). Biodegradable self-assembled nanoparticles are engineered for targeted delivery of anticancer drugs, serving as a vehicle to ferry large doses of chemotherapeutic agents into malignant cells while sparing healthy cells (Sinha et al., 2006). Size-specific anticancer drug nanostructures are able to penetrate tumor microvasculatures via pores ranging from 100 – 1000nm, but are kept out of healthy tissues such as the heart, brain and lung by tight intercellular junctions measuring less than 10nm (Hughes, 2005).

Bisht et al. (2008) synthesised and studied the drug delivery efficacy of amphiphilic nanoparticles comprising of N-isopropylacrylamide, methylmethacrylate, and acrylic acid. The nanoparticles readily encapsulate water insoluble drugs such as rapamycin for cancer therapy and showed favourable in-vivo pharmacokinetics of the drug, with no apparent systemic toxicities from oral administration in mice (Bisht et al., 2008). Anticancer drugs such as paclitaxel and tamoxifen have been encapsulated in poly(lactic-co-glycolic) acid and polycaprolactone nanoparticles with high

encapsulation efficiency (90 – 100 %), and respectively demonstrated enhanced anti-tumoral efficacy and increased accumulation in tumour with extended systemic presence compared to free drug (Kumari et al., 2009).

Peptide drugs and proteins previously faced limitations in oral delivery due to epithelial barriers and susceptibility to enzymatic degradation in the gastrointestinal tract. They are now encapsulated in nanocarriers, which serve as protective vehicles, enhancing the oral bioavailability of peptides such as insulin and salmon calcitonin (Ochekpe et al., 2009). Insulin has been encapsulated in biopolymeric nanoparticles such as poly(lactic-co-glycolic) acid, polycaprolactone and chitosan, all of which showed high encapsulation efficiency (75 – 96 %), preserved biological activity and enhanced intestinal absorption (Kumari et al., 2009). Damgé et al. (1997) demonstrated the protective effect of poly-(alkyl cyanoacrylate) nanocapsules in oral administration of insulin in a rat study. Insulin-loaded nanospheres with mean size of 145nm were dispersed in oily medium containing surfactants and administered perorally in streptozotocin-induced diabetic rats. The authors observed a 50% decrease of fasted glycemia from the second hour up to 10-13 days, indicating the sustained release and bioactivity of the nano-encapsulated insulin (Damgé et al., 1997). Elsayed et al. (2009) recently assessed the storage stability of insulin-loaded chitosan nanoparticle preparation using streptozotocin-diabetic rats as in-vivo models. The authors found insignificant difference in pharmacological activity between the groups taking fresh preparation and stored preparation, indicating the storage biological stability of the nano-encapsulated insulin (Elsayed et al., 2009).

In tissue engineering and regenerative medicine, microparticles with diameters ranging from 1 to 10 μm, are incorporated into porous scaffolds to deliver encapsulated bioactive agents to incorporate osteoconductive materials in the system (Oliveira & Mano, 2011). Other biologically active agents which have been encapsulated into microparticles include hormones such as calcitonin (Woo et al., 2008).

(b) Liposomes

Stealth liposomes are being studied as carriers for hydrophilic anticancer drugs such as doxorubicin and mitoxantrone (Samad et al., 2007). In addition to extended systemic circulation time and reduced toxicity, liposomes increase efficacy of cancer therapy by facilitating preferential localisation of anti-cancer drugs in tumors via increased endothelial permeability and reduced lymphatic drainage, leading to enhanced permeability and retention effect (Wu et al., 2007). Anti-cancer drug doxorubicin was encapsulated in pH-sensitive micelles with acid-labile acetal groups on the core-forming dendrimer periphery, designed for targeted release in acidic environment (Cui et al., 2008).

Liposomes are modified to be degraded in low pH or fuse with the endosomal or lysosomal membranes (Cohen-Sela et al., 2007). Such modifications allow for selective release of encapsulated compounds, by modulating the chemical properties of nanoparticles to suit the environment in which it is to resist or rupture. Surface modification by attaching polyethylene glycol units extends systemic circulation of liposomes, while conjugation to ligands adapts liposomes to targeted delivery and confers stability (Ochekpe et al., 2009). Liposomes are also being developed to cross the blood brain barrier, which has challenged central nervous system drug delivery

with its relatively impermeable endothelial cells consisting of tight junctions and specific receptor-mediated transport systems (Mohanraj & Chen, 2006).

(c) Carbon Nanotubes

Carbon nanotubes (CNTs) are excellent sub-cellular delivery vehicles which are functionalised with bioactive peptides, proteins, nucleic acids and drugs (Bianco et al., 2005). CNTs comprise of single-walled nanotubes (SWCNT) and multi-walled nanotubes (MWCNTs). CNTs drug delivery system is highly promising due its physicochemical and structural properties which provide advantages in terms of safety and monitoring means. CNTs have among the safest chemical composition of purely carbon; unique one-dimensional structure and tunable length ideal for investigating size and shape effects in-vivo; and intrinsic spectroscopic properties i.e. Raman and photoluminescence which provide valuable means for monitoring in-vivo behavior and drug delivery efficacy (Liu et al., 2008). As such, CNTs have become highly relevant in oncology and imaging applications.

CNT have been studied as a template for presenting bioactive peptides to the immune system (Bianco et al., 2005). Drugs are internalised into the cells at the targeted sites either with or without the carriers. CNTs are internalised via endocytosis or endocytosis-independent pathways i.e. via insertion and diffusion through the lipid bilayers of cells (Tran et al., 2009). Nanotubes functionalised with antibiotics such as amphotericin B have been reported to be easily internalised into mammalian cells without toxic effects and preserved high antifungal activity against pathogens (Bianco et al., 2005).

CNTs plays a big role in the progress of cancer treatment, providing powerful targeting ability and potential for large cytotoxic payload, hence dramatically enhancing the efficacy of conventional pharmaceuticals (Cohen-Sela et al., 2007). The overall efficacy has been found to be enhanced by the intrinsic spectroscopic property of CNTs in the near IR (NIR) region which effectively destroyed cancer cells in-vitro and efficient drug-loading attributable to its high surface area (Liu et al., 2008). The size of CNTs can be modified to specifically penetrate the pores of blood vessels in tumor tissues which, at 100 – 700 nm are distinctively larger than the pores of blood vessels in healthy tissues at 2 – 6 nm (Tran et al., 2009).

One of the pioneering reports of in-vivo efficacy of CNTs as drug delivery vehicles for tumour treatment in mice was reported by Liu et al (2008). The authors studied the SWCNT delivery of paclitaxel, a common cancer chemotherapy drug. The PolyEthyleneGlycolated (PEGylated) SWCNTs functionalised with paclitaxel displayed strong therapeutic efficacy via tumour suppression achieved at low injected drug dose, suggesting the potential of CNTs in future cancer therapeutics. In another study, the strong absorption of SWCNT in the NIR region was used to selectively heat up cancer cells in-vivo. SWCNTs are functionalised to selectively bind to receptors which are over-expressed in cancer cells, thus allowing selective heat treatment on cancer cells, sparing the surrounding normal cells (Wong et al., 2005). This hyperthermia treatment of cancer uses controlled, elevated heat to kill cancer cells, which are more susceptible to heat compared to normal cells, due to impaired heat dissipation system (Tran et al., 2009).

(d) Nanofibers

Nanofibers produced via electrospinning forms porous network with very large surface area-to-volume ratio, thus rendering nanofibers suitable agents for controlled drug release and as scaffolds for tissue engineering. Electrospun nanofibers have been explored as potential biomedical device and delivery carriers for bioactive agents such as antibiotics, anti-tumor agents, proteins and plasmid DNA (Xu et al., 2006; Kim et al., 2007; Cui et al., 2008).

Polysaccharides such as chitosan with intrinsic bacteriostatic activity in addition to advantageous biological properties such as hemostatic activity, biodegradability and non-toxicity, have potential to be developed into revolutionary nanofiber wound-care applications which expedites healing while minimising infection risks (Yacoby & Benhar, 2008). The hollow morphology of nanofibers contributes potential encapsulating and sustained release properties (Burke & Luzhansky, 2007). Xu et al. (2006) reported that 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU)-loaded poly(ethylene glycol)–poly(l-lactic acid) (PEG–PLLA) diblock copolymer fibers would exhibit anti-tumour activity over 72 hours while pristine BCNU lost the activity after 48 hours (Xu et al., 2006). The embedding of the anti-cancer drug within polymer fibers preserved the bioactivity of the drug and the amount of drug loaded were modified to control drug release (Xu et al., 2006; Sill & von Recum, 2008).

Additionally, electrospun nanofibrous have been found to be able to provide controlled delivery of hydrophilic and hydrophobic drugs, of which their release can be tailored by modulation of nanofibrous scaffold's morphology, porosity, and composition (Vasita & Katti, 2006). Immediate release nanofibers are created by water-soluble

polymers, enteric-release nanofibers by enteric polymers such as methacrylic acid copolymers and sustained-release nanofibers by polylactic acid or polyacetate polymers (Burke & Luzhansky, 2007). Verreck et al. (2003) studied the delivery of hydrophobic drugs intraconazole and ketanserin incorporated as amorphous nanodispersion on polyurethane nanofibrous scaffolds. Nanofibers have also been electrospun from water-soluble polymers such as polyvinyl alcohol, whereby the fiber/film-forming ability and easy cross-linking was useful in developing wound treatment (Yacoby & Benhar, 2008). Electrospun poly(vinyl alcohol) nanofibers, with inherent non-toxicity and good biocompatibility, were studied for controlled delivery of ketoprofen, a non-steroidal anti-inflammatory drug (NSAID) (Kenawy et al., 2007). The cross-linked poly(vinyl alcohol) nanofibers released 20.04 % of its drug content within two hours and a total of 69.18 % of its drug content within two weeks.

In addition to drugs, nanofibers have also been formulated to encapsulated biological material such as DNA, proteins, enzymes and other small molecules (Salalha et al., 2006). Plasmid DNA and bacteria were found to be released intact and viable from electrospun nanofibers, suggesting the potential of nanofibers for applications in gene therapy (Salalha et al., 2006).

Nanolaminates thin films of nanofibers formed by multiple nanolayers of carbohydrates, proteins, charged lipids or colloidal particles (Weiss, Takhistov & McClements, 2006). Nanolaminates containing bioactives agents such as anti-browning agents, enzymes, colours, flavours, antioxidants and nutrients are coated on the charged surface of the product to be laminated (Decher & Schlenoff, 2003). It is

mainly applied in food packaging as edible coating that prevents moisture, lipids and gas diffusion in meats, fruits, vegetables and confectionery (Kuan et al., 2012).

1.2 AGROWASTE AND PROBIOTICS AS MODELS FOR ELECTROSPINNING FUNCTIONAL NANOFIBERS

1.2.1 INTRODUCTION

Agrowastes are wastes generated from agricultural and agro-based activities, which are carried out on more than 6 million hectares of land in Malaysia. The abundance and low cost of agrowaste has promoted much research in converting the waste into functional products and reduce waste disposal issues in agriculture industry. With their unique nutrient composition and physicochemical make-up, agrowastes have the potential to be recycled into profit-generating products such as organic fertilizers, fermentation medium and bio-fibers with extensive application in the construction, textile, paper and even food industries. Prakongpan et al. (2002) incorporated dietary fiber and cellulose from pineapple core into beef burgers to increase the weight after cooking and pineapple core fiber was found to improve the texture of the product. The function of agrowaste as dietary fibers may even be extended to serve as prebiotics, or substrate for beneficial bacteria called probiotics which promote gastrointestinal health.

Probiotics are "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host", according to the World Health Organization. Probiotics confer health benefits by promoting the proliferation of beneficial microflora in the gastrointestinal microenvironment (Lye et al. 2009). One of the most common probiotic strains are of the genus *Lactobacillus*. Species of lactobacilli that

have been isolated from the human gastrointestinal tract include the *L. acidophilus, L. fermentum, L. plantarunm, L. brevis, L. casei* and *L. leichmanii. L. acidophilus* is able to survive in low pH environments of pH 4 and below, as well as the harsh conditions of the gastrointestinal tract en route to the small intestines where it proliferates. *L. acidophilus* is a facultative anaerobe which produces only lactic acid as the sole metabolite at optimum growth temperature of 37 to 42˚C. The bacterium is physically characterised as a rod-shaped of 2 to 10 µm. The health promoting effects of probiotics are well established and probiotics have been increasingly used as functional adjuncts such as soymilk, fruit juices, meat derivatives, cereal-based foods in addition to conventional probiotic supplements, such as cultured milk and freeze-dried powder capsules. The therapeutic dose of probiotics via oral administration is in excess of 10^9 colony forming units (cfu)/day.

1.2.2 AGROWASTE FROM OIL PALM AND SOY (OKARA) AS FUNCTIONAL FIBER SOURCE

Indonesia and Malaysia are the largest oil palm producers, while the U.S.A. and Brazil are the largest producers of soybean crop. These agriculture industries are major contributors to biomass waste. Approximately two hundred billion tonnes of lignocellulosic wastes being produced annually, creating environmental concerns (Mohanty et al., 2000). These wastes are rich in fibers and nutrients, making them potentially valuable using proper recycling technologies. Only 10% of the oil palm crop is economically valuable, used for the production of palm oil, while the remaining 90% constitutes of oil palm trunks (OPT), oil palm fronds (OPF), empty fruit bunches (EFB) and palm pressed fibers (PPF) which are disposed as agriculture waste or are recycled to other industries (Ratnasingam et al., 2008). Oil palm trunk (OPT) contains

30% lignocelluloses which are constituted of cellulose, hemicelluloses and lignin (Ratnasingam et al., 2008). Oil palm frond (OPF) contains high levels of holocellulose (82.2 %) and α-cellulose (47.6%), as well as lignin (15.2%) (Wan Rosli et al., 2007). Okara, the main solid waste from soy and tofu processing industry, is constituted of up to 49 % fiber (Prestamo et al., 2007). Conventionally, most oil palm and soy biomass are being converted to biofuel substrate and animal feed. With further research and development, the exploitation potential of these agriculture waste remains expandable as the material is locally abundant, renewable and relatively economical.

Oil palm and soy agrowastes are good sources of fiber which includes cellulose, hemicelluloses, lignin, pectin, gums and other polysaccharides. Soluble and insoluble dietary fiber have been found to confer a wide range of health benefits, including general reduction in risk of civilisation diseases such as cardiovascular diseases, gastrointestinal diseases and obesity. Dietary fiber is also noted as prebiotics which are substrates for the growth and proliferation of beneficial gut bacteria, hence enhancing the beneficial properties of probiotics. Thus, the market demand for palatable high-fiber products as functional foods with a low glycemic index and hypocholesterolemic properties is increasing. However, high-fiber content in food is often associated with undesirable sensory properties due to the inherent properties of fibers being coarse and grainy. The food industry has developed compound coatings which can effectively mask fibrous mouth feel associated with dietary fiber. However, compound coatings are essentially made up of fats and carbohydrates, which increase the caloric value of the food. The diminutisation of fibers to nano-size can reduce the undesirable organoleptic properties and eliminate the need for additional processing

or high calorie additives which will negate the beneficial properties of the fibers. Apart from supplementing dietary fiber, the oil palm and soy agrowaste are also processed to form composites in textile and construction materials.

1.2.3 FUNCTIONAL PROPERTIES OF PROBIOTICS

The physiological benefits of consuming probiotics are well documented, with the most evidence and extensive use in the maintenance of gastrointestinal health. Past studies have shown the therapeutic activities of various strains probiotics in gastrointestinal disturbance such as diarrhea, irritable bowel syndrome, rotavirus, *Helicobacter pylori* and *Clostridium difficile* infections of the gut (Guandalini et al., 2000; Cats et al., 2003; Whorwell et al., 2006; McFarland, 2009; Moayyedi et al., 2010).

A comprehensive meta-analysis conducted by McFarland (2006) revealed that probiotics resulted in significant prevention of antibiotic-associated diarrhea and *C. difficile* infections compared to placebo groups, in 25 randomised controlled trials involving 2810 subjects. Similarly, a systematic review by Moayyedi et al. (2010) revealed significant alleviation of irritable bowel syndrome symptoms such as pain and flatulence compared to the placebo groups in all 19 randomised controlled trials involving 1650 patients. *L. acidophilus* and *L. casei* Shirota showed antagonistic action against *Helicobacter pylori* (Cats et al., 2003) while *L. rhamnosus* GG and *B. lactis* BB-12 were effective for prevention and treatment of acute diarrhoea caused by rotaviruses in children (Guandalini et al., 2000). The mechanisms of action by probiotics in exerting these effects include competitive exclusion of pathogenic bacteria for nutrients and adhesion sites (Rolfe, 2000), production of antimicrobial